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SUPPORTING INFORMATION

Conformational-Specific Self-Assembled Peptides as Dual-Mode, Multi-target Inhibitors and Detectors for Different Amyloid Proteins

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Materials and Methods

1 Reagents

1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, ≥99.9%), 10 mM PBS buffer (pH=7.4), and thioflavin T (ThT, 98%) were purchased from Sigma-Aldrich (St.Louis, MO). Dime-thyl sulfoxide (DMSO, ≥99.9%) was purchased from ATCC Inc (Manassas, VA). Human IAPP (1-37) (≥95.0%) were purchased from Bachem Americas Inc. (Torrance, CA). Hexapeptides (>95% purity) were synthesized by Selleck-chem Inc. (Houston, TX). All other chemicals were of the highest grade available.

2. Instruments

ThT fluorescence assay was carried out by LS-55 fluorescence spectrometer (Perkin-Elmer Corp., Waltham, MA). AFM was carried out by Nanoscope III multimode scanning probe microscope (Veeco Corp., Santa Barbara, CA) equipped with a 15 μm E scanner. CD was carried out by J-1500 spectropolarimeter (Jasco Inc., Japan). MTT cytotoxicity was carried out by Synergy H1 microplate reader (BioTek, Winooski, VT). Cell LIVE/DEAD assay was carried out by EVOS XL core inverted microscopy (Advanced Microscopy Group, Bothell, WA). Pictures of paper sensor results were taken by using the AzureTM C300 Biosystem (Azure Biosystems Inc, Dublin, CA). SPR was carried out by A custom-built four-channel SPR sensor based on wavelength interrogation.

3 Peptide Purification

hIAPP was obtained in a lyophilized form and stored at -20 ℃ as arrived. To obtain the monomeric hIAPP solution, 1.0 mg of original hIAPP powder was dissolved in HFIP for 2 h, and sonicated for 30 min to remove any pre-existing aggregates or seeds, then centrifuged at 14,000 rpm for 30 min at 4 °C. 80% of the top hIAPP solution was then extracted, sub-packaged, frozen with liquid nitrogen, and then dried with a freeze-dryer. The dry hIAPP powder was lyophilized at -80 °C and used within 1 week. Same purification procedure is applied to each hexapeptide as well.

4 Self-assembly of Hexapeptides

Right before the beginning of each incubation, around 0.14mg purified lyophilized hexapeptide powder was predissolved in 10mM NaOH then dissolved into 2mL 10nmM PBS solution to achieve 100 uM hexapeptide solution. The exact amount of hexapeptide powder used in each group is based on relative molecular weight ((1)737.92, (2)764.90, (3)741.86, (4)694.90, (5)638.78 and (6)704.79). This solution was then centrifuged at 14,000 rpm for 30 min at 4 °C to dissemble any pre-formed nuclei, and 80% of the top solution was removed for further incubation. All hexapeptides solutions were incubated at 37 °C.

5 Peptide Incubation

Immediately before use, 0.2 mg of purified hIAPP pow-der was aliquoted in 20 μL DMSO and sonicated for 1 min to obtain a homogenous solution. The initiation of hIAPP (25uM, containing 1% (v/v) DMSO) aggregation in solution was accomplished by adding that 20uL DMSO-hIAPP solution to 2ml of 10 mM PBS buffer. This solution was then centrifuged at 14,000 rpm for 30 min at 4 °C to re-move any existing oligomers, and 80% of the top solution

was removed for further incubation. We used the same protocol to prepare each mixed hIAPP:hexapeptide solutions, with the only difference in changing the initial amount and ratio of purified peptide powder. The amount of hexapeptide in each mixed group is deter-mined by concentration ratios (1:2, 1:4 and 1:6) and molecular weight of each hexapeptide. All the solutions were incubated at 37 °C.

6 Thioflavin T (ThT) Fluorescence Assay

A ThT solution (2 mM) was prepared by adding 0.032 g of ThT powder into 50 mL of DI water. The resulting 250 μL of the 2 mM ThT solution was further diluted with 50 mL of Tris-buffer (pH = 7.4) to a final concentration of 10 μM. At each collection point, 60 μL of incubation solution was put into 3 mL of 10 μM ThT-Tris solution. All measurements were carried out in aqueous solution using a 1 cm × 1 cm quartz cuvette. ThT fluorescence emission wavelengths were recorded between 460 and 510 nm with an excitation wavelength of 450 nm. Each experiment was repeated by at least three times, and each sample was tested in quintuplicate.

7 Tapping-mode AFM

A 20 μL sample solution from the endpoint of each hexapeptide self-assembly and hIAPP-hexapeptide coincubation group was taken for AFM measurement to rep-resent aggregates morphology. Incubation solution was deposited onto a freshly cleaved mica substrate for 1 min, rinsed three times with 50 mL of deionized water to re-move salts and dried with compressed air for 5 min be-fore AFM imaging. Commercial Si cantilevers (NanoScience) with an elastic modulus of 40 N m−1 were used. All images were acquired as 512-pixel images at a typical scan rate of 1.0−2.0 Hz with a vertical tip oscillation frequency of 250−350 kHz. Representative AFM images were obtained by scanning at least six different locations of different samples.

8 Circular Dichroism Spectroscopy (CD)

The secondary structures of hexapeptide assembly in solution were examined by CD spectroscopy with a J-1500 spectropolarimeter (Jasco Inc., Japan) using a continuous scanning mode at room temperature. At each data collection time point, 160uL of peptide solutions were placed into a rectangular quartz cuvette of a 1mm path length without dilution. The spectra were scanned between 250 and 190 nm at a 0.5 nm resolution and 50 nm/min scan rate. All spectra were corrected by subtracting the baseline and averaged by three successive scans for each sample.

9 Cell Culture

Rat insulinoma (RIN-m5F) cells (ATCC, Manassas, VA) were used as model pancreatic β-cells, and cultured in 75 cm2 T-flasks in sterile-filtered RPMI-1640 medium (ATCC, Manassas, VA) containing 10% fetal bovine serum (ATCC, Manassas, VA) and 1% penicillin/streptomycin (ATCC, Manassas, VA). Flasks were incubated in a humidified incubator with 5% CO2 at 37°C. Cells were then cultured to confluence and harvested using 0.25% Trypsin-EDTA (1x) solution (Lonza, Walkersville, MD). Cells were count-ed using a

hemocytometer and plated in a 96-well tissue culture plate at 50,000 cells per well in 100 μL medium, which allow them to attach inside the incubator for 24 hours.

10 MTT Cell Toxicity Assay

MTT-based cell toxicity assays were performed to assess the cytotoxicity of hIAPP and hexapeptide assemblies. In hexapeptide self-assembly toxicity test, 96-well plate with cells was split into 8 groups, with each group containing 6 replicates. The first group containing cells only in medium was used as a positive control. The second group containing 2% DMSO was used as an addition-al control for evaluating the effects of the DMSO on cells. DMSO-hexapeptide (1-6) solutions diluted by cell medium were respectively added to the groups of 3-8 to achieve the 100μM hexapeptide concentration. In hIAPP-hexapeptide toxicity test, 96-well plate with cells was split into 9 groups, with each group containing 6 replicates. The first and second group were still positive control and DMSO control. DMSO-hIAPP diluted by cell medium was added to the group 3 to achieve a 25 μM hIAPP concentration. Then DMSO-hIAPP solutions are mixed with DMSO-hexapeptide (1-6) solutions in cell medium respectively and then were added to group 4-9 to achieve a 25 μM hIAPP and 50 μM hexapeptide mixture cell medium solution in each group. Then we repeat the 96-well plate hIAPP-hexapeptide (1-6) solutions preparation by respectively double and triple hexapeptide amount to achieve a 25 μM hIAPP and 100 μM hexapeptide mixture and a 25 μM hIAPP and 150 μM hexapeptide mixture.

For hexapeptide self-assembly groups, the cells were then incubated for another 120 hours (the same medium changed after 60h), while for hIAPP-hexapeptide groups, the cells were then incubated for another 24hours. After cell incubation finish, they are assessed for cell toxicity using the Vybrant MTT Cell Proliferation assay kit (Life Technologies, Grand Island, NY). A 12 mM MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was prepared by dissolving 5 mg MTT per 1 ml of sterile PBS (phosphate buffered saline). 15.3 μL of this MTT solution was added to each well. The cells were incubated for 4 hours at 37°C to convert MTT to formazan crystals. Cells were then removed from the incubator, and all but 50 μL of medium was removed from each well. Formazan crystals formed at the bottom of each well were dissolved by adding 100 μl of DMSO per well and thoroughly mixed. Cells were incubated for an additional 10 minutes at 37°C and mixed again to ensure formazan was fully dissolved. Plates were placed in a Synergy H1 microplate reader (BioTek, Winooski, VT) and absorbance was read at 540 nm to determine formazan content. The absorbance of positive control wells was averaged and subtracted from all other samples to eliminate back-ground effect. Sample absorbance was then compared to the control groups to determine cell viability.

11 Cell Live and Dead Assay

Cell live and dead assay were conducted using the Live/Dead Viability/Cytotoxicity Assay Kit. Following incubation with various test agents, the solutions were re-moved and 100 □L of viability assay solution was added. The assay solution consisted of 4 □M EthD-1 solution and 2 M calcein AM.

12 MD Simulation Protocol

To better explain the like-interact-like inhibition effects of hexapeptides on hIAPP and Aβ (PDB:2BEG), MD (molecular dynamics) simulation is applied. We select the hexapeptides (HP1, HP2, HP3, HP6) which have inhibition effects both on hIAPP and Aβ from the experiment result showed above. The second structure construction of hexapeptides is via the scripts we create in our group. The structure of hIAPP and Aβ derived from Tycko's (Luca et al. 2007) and Riek's (Luhrs et al. 2005) group dissolved by NMR device. The first step is by using the PatchDock and Fire-Dock web docking tools to find the favorite sites of hexpeptides on amyloid proteins. Each binding system is solved by water molecules in the boundary condition of at least 15 Å from solute. Na+ and Cl- counterions are added to neutralize system and mimic 15 mM ionic strength. The all-atom MD simulation processes of all models are carried out in NAMD 2.19 program and CHARMM27 force field with the CMAP correction(Phillips et al. 2005) is used to characterize each system. The temperature and the pressure are separately maintained at 310 K and 1atm in NPT ensemble by the Langevin method. Short-range van der Waals (vdW) and long-range electrostatic forces are introduced via switch functions and force-shifted methods with the cutoff of 14 Å. We use in-house TCL codes to analyze the MD simulation result.

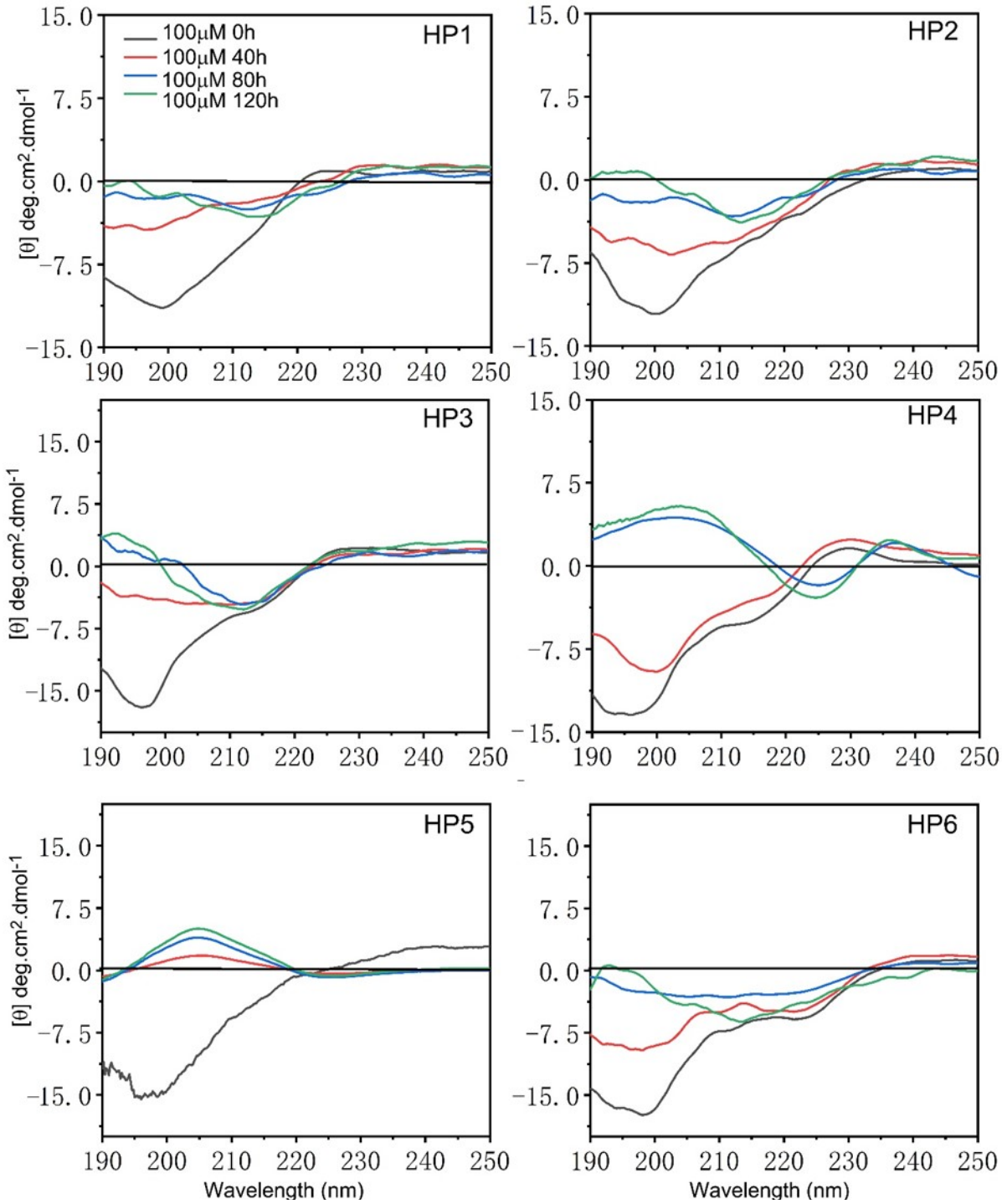


Figure S1. Time-dependent far-UV CD spectra to characterize the secondary structure changes of six SAPs (100 μM) during 120 h incubation.

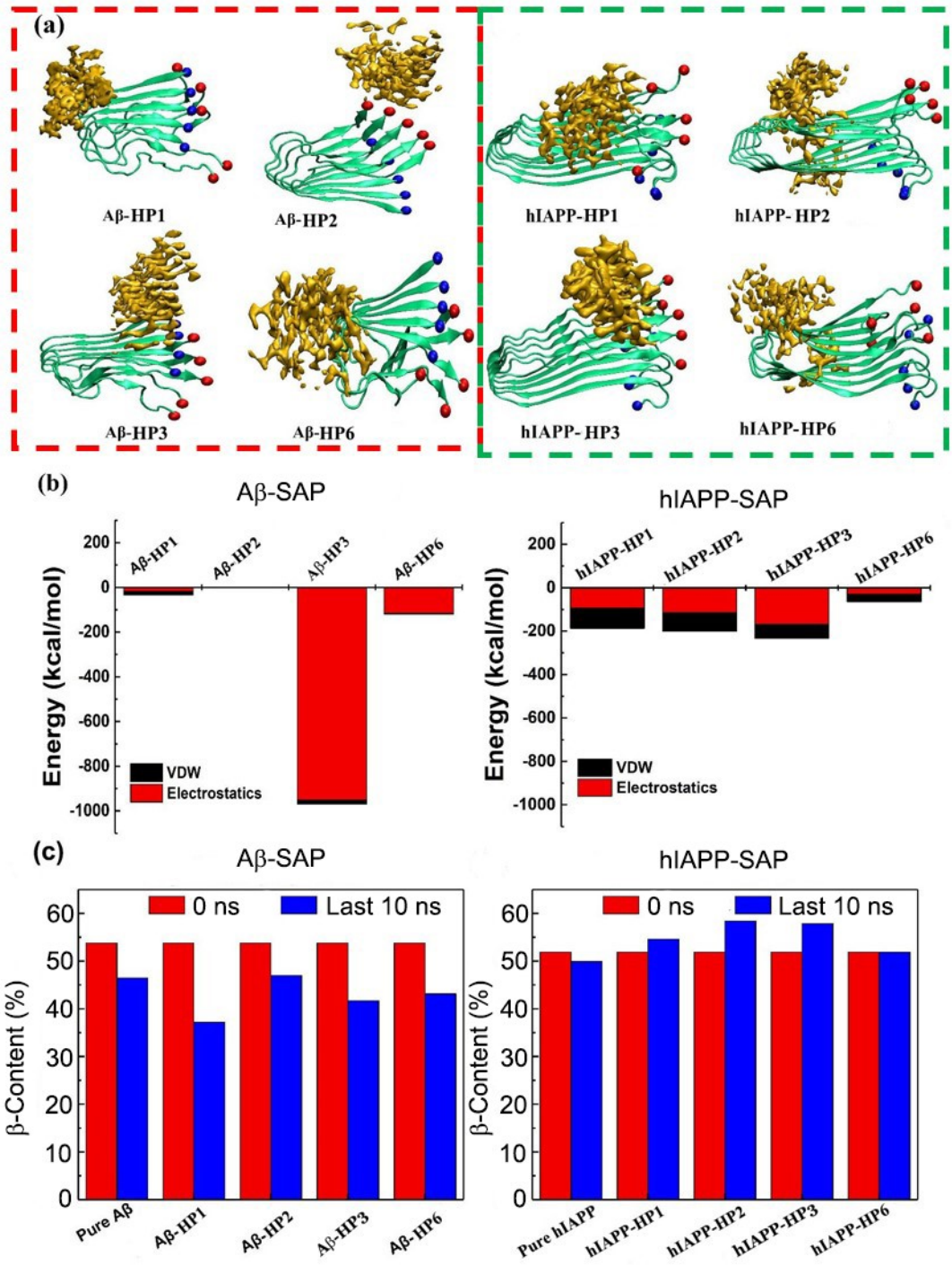


Figure S2. Structure and interactions of between SAPs and Aβ and between SAP and hIAPP by molecular dynamics simulations. (a) Preferential binding positions of four SAPs (HP1, HP2, HP3, and HP6) around Aβ and hIAPP pentamers. (b) Nonbonded interaction energy between four SAPs and Aβ or hIAPP, consisting of electrostatic (red) and VDW (black) contributions. (c) β-content changes of Aβ and hIAPP pentamers before and after binding to SAPs.

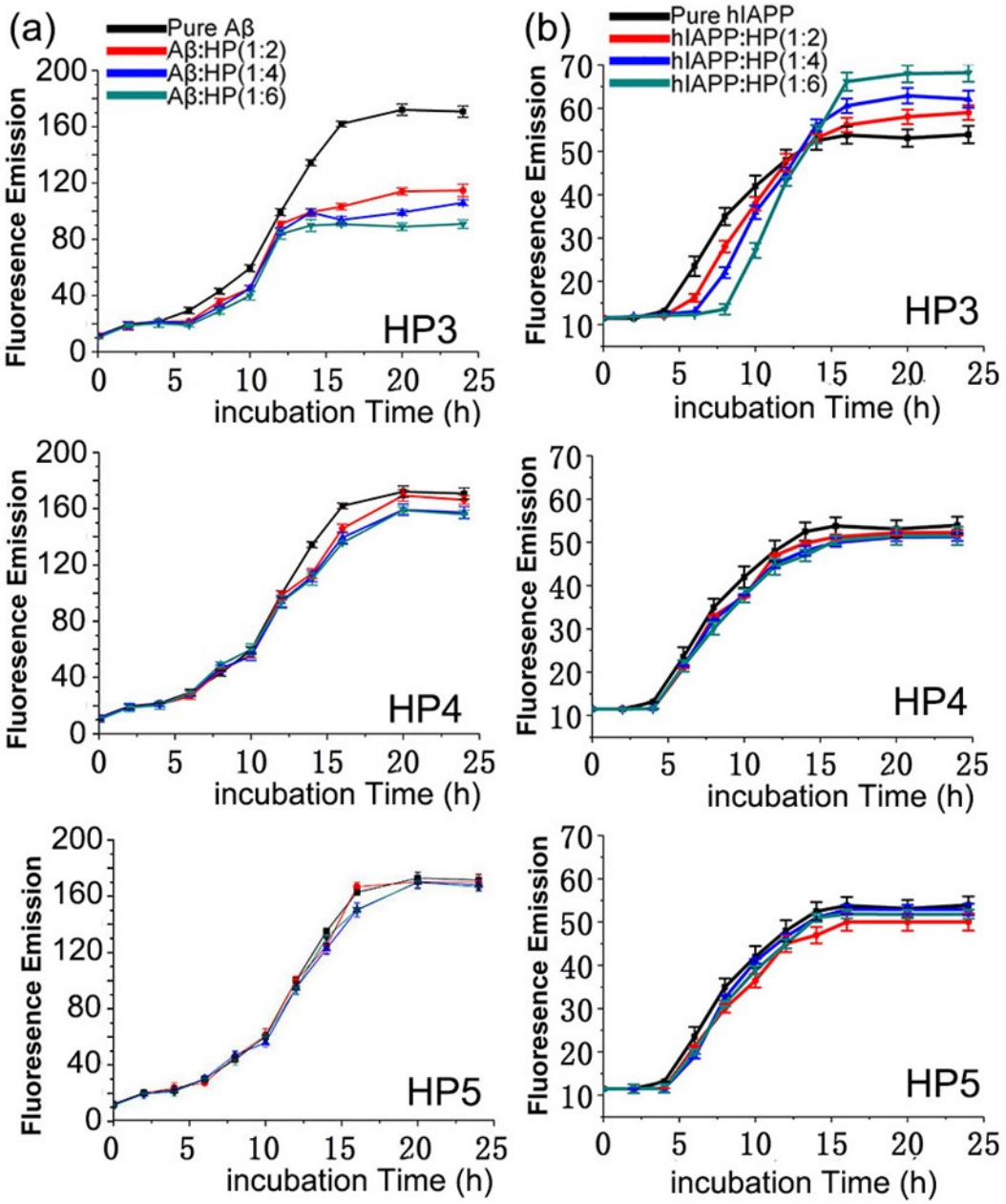
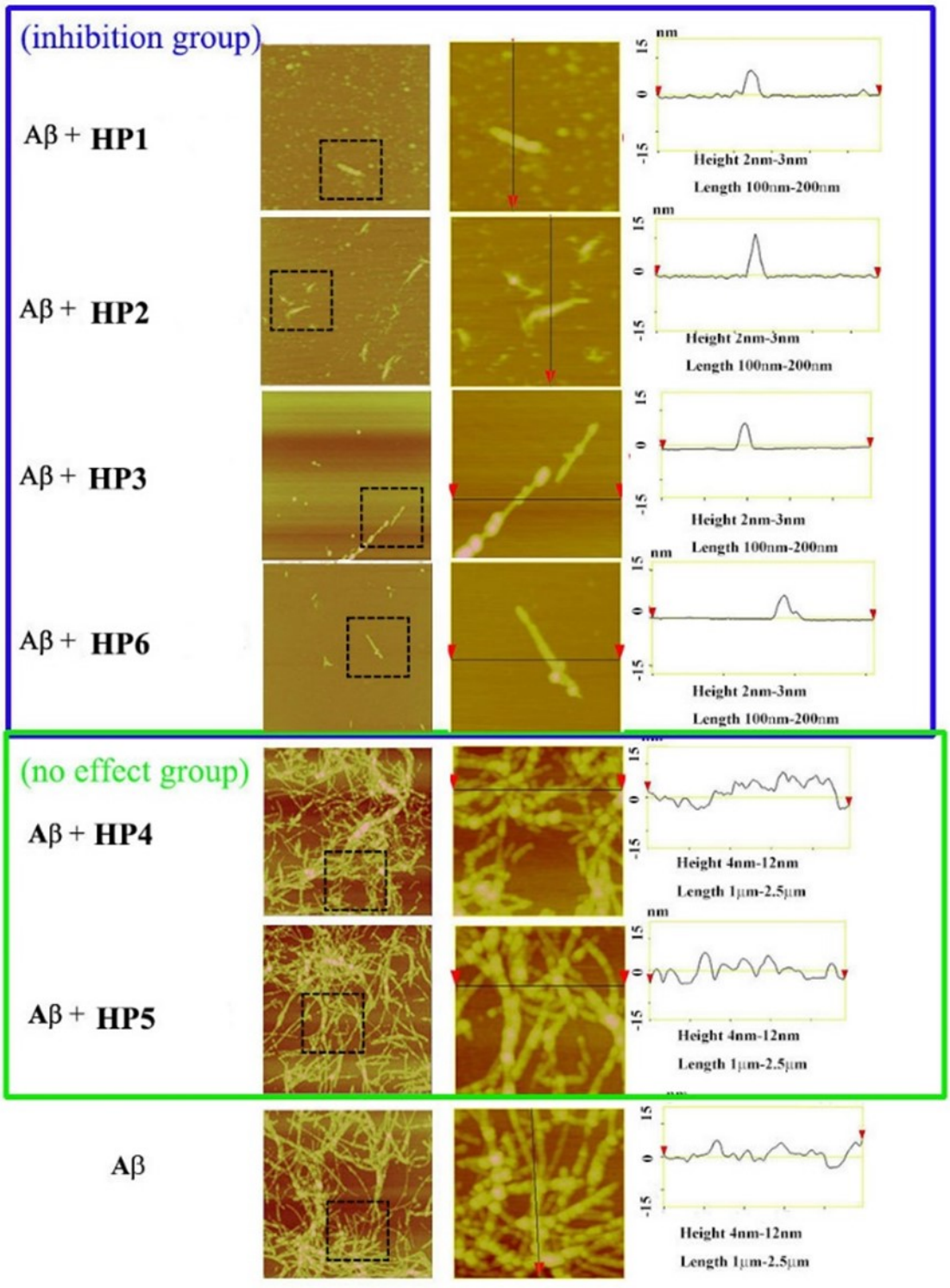


Figure S3. Time-dependent ThT fluorescence curves for (a, left panel) Aβ□(25 μM) and (b, right panel) hIAPP (25 μM) aggregation in the absence (control) and presence of HP3, HP4, and HP5 at different molar ratios of amyloid/SAP (1:1, 1:2, and 1:6). Error bars represent the average of three replicate experiments.


(inhibition group)
Aβ + HP1
Aβ + HP2
Aβ + HP3
Aβ + HP6
nm
15
0
-15
Height 2nm-3nm
Length 100nm-200nm
(no effect group)
Aβ + HP4
Aβ + HP5
Height 4nm-12nm
Length 1μm-2.5μm
Aβ

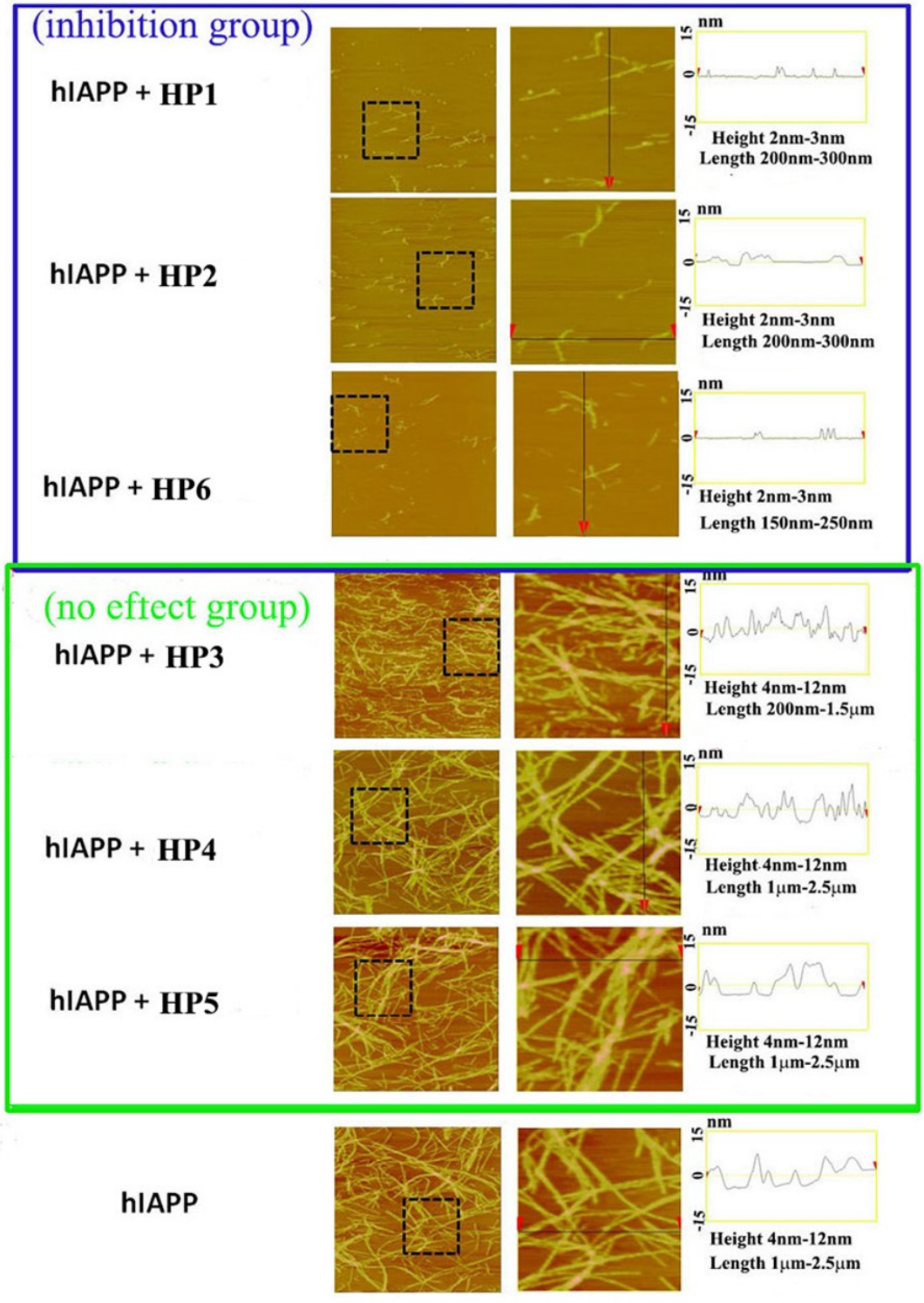


Figure S4. Representative AFM images and morphological analysis of co-incubation of (a) Aβ (25 μM) and (b) hIAPP (25 μM) with SAPs (HP3, HP4, or HP5 at 150 μM) at 24h.

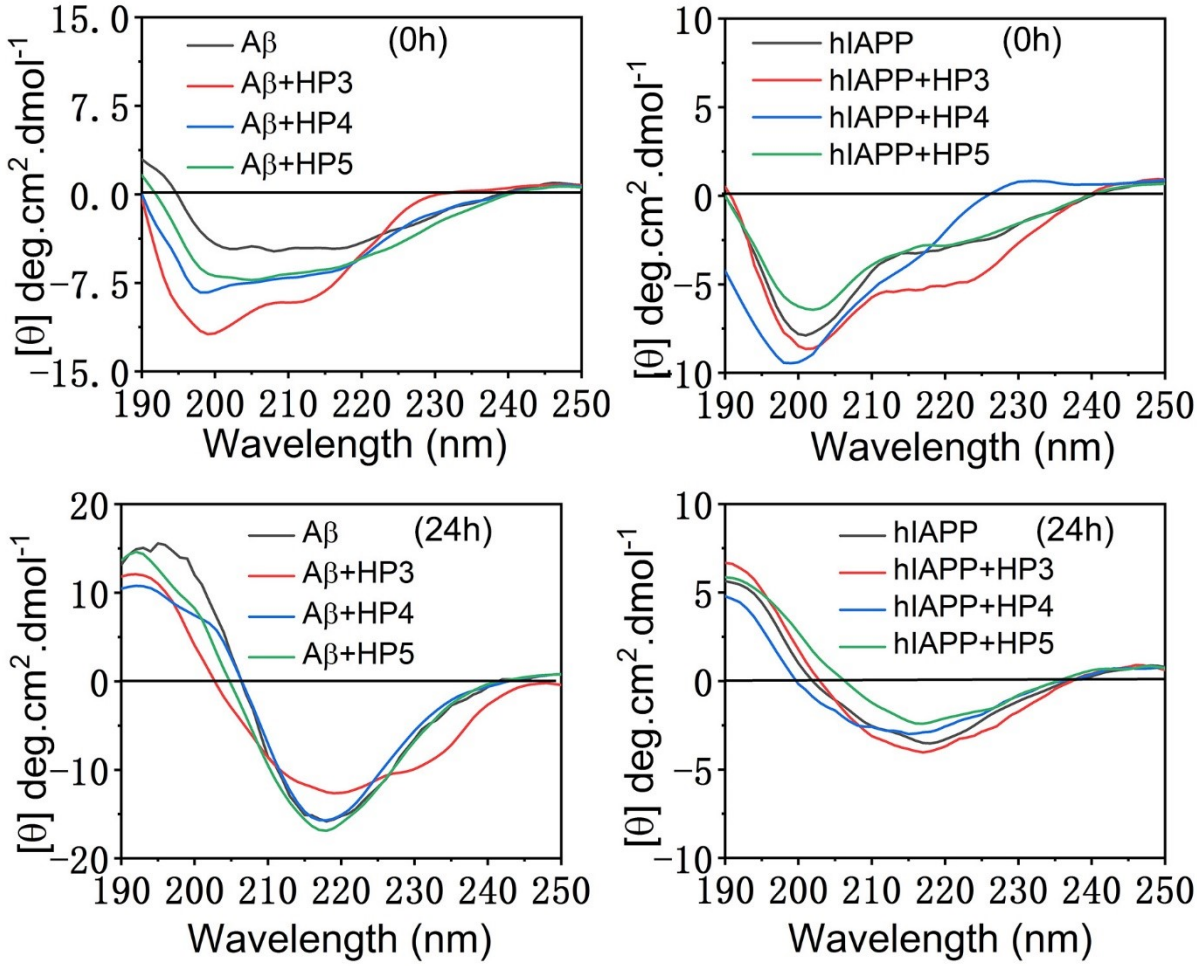


Figure S5. CD spectrum for amyloid and SAPs at the molar ratios of amyloid:SAP=1:6.

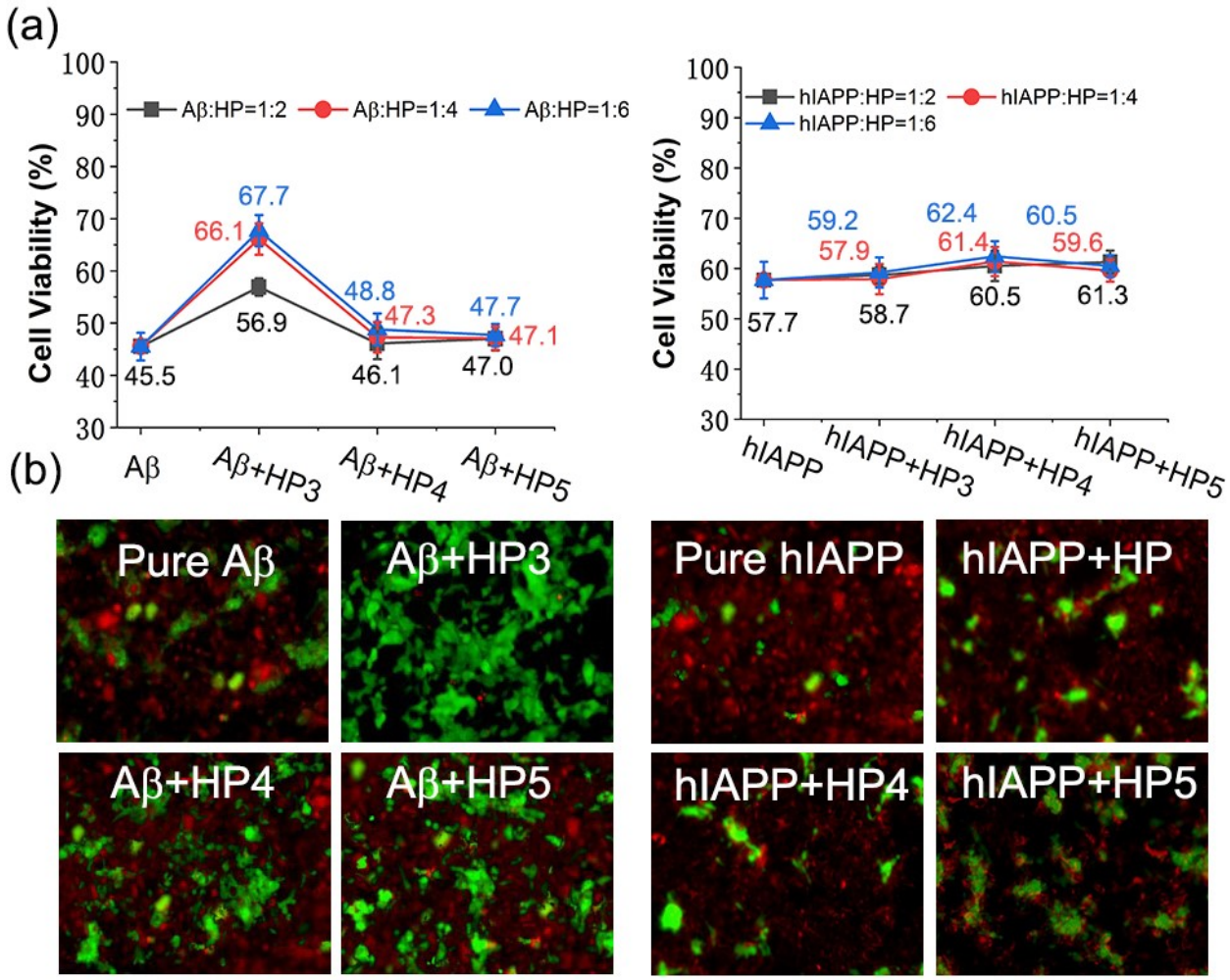


Figure S6. Effects of CTIYWG (HP3), VYIMIG (HP4) and CTVFIG (HP5) on the Aβ-induced SH-SY5Y and hIAPP-induced RIN-m5F toxicity at different amyloid (25 μM):SAP (25, 100, and 150 μM) ratios using (a) MTT assay and (b) amyloid (25 μM):SAP (150 μM) ratio using live/dead cell assay.

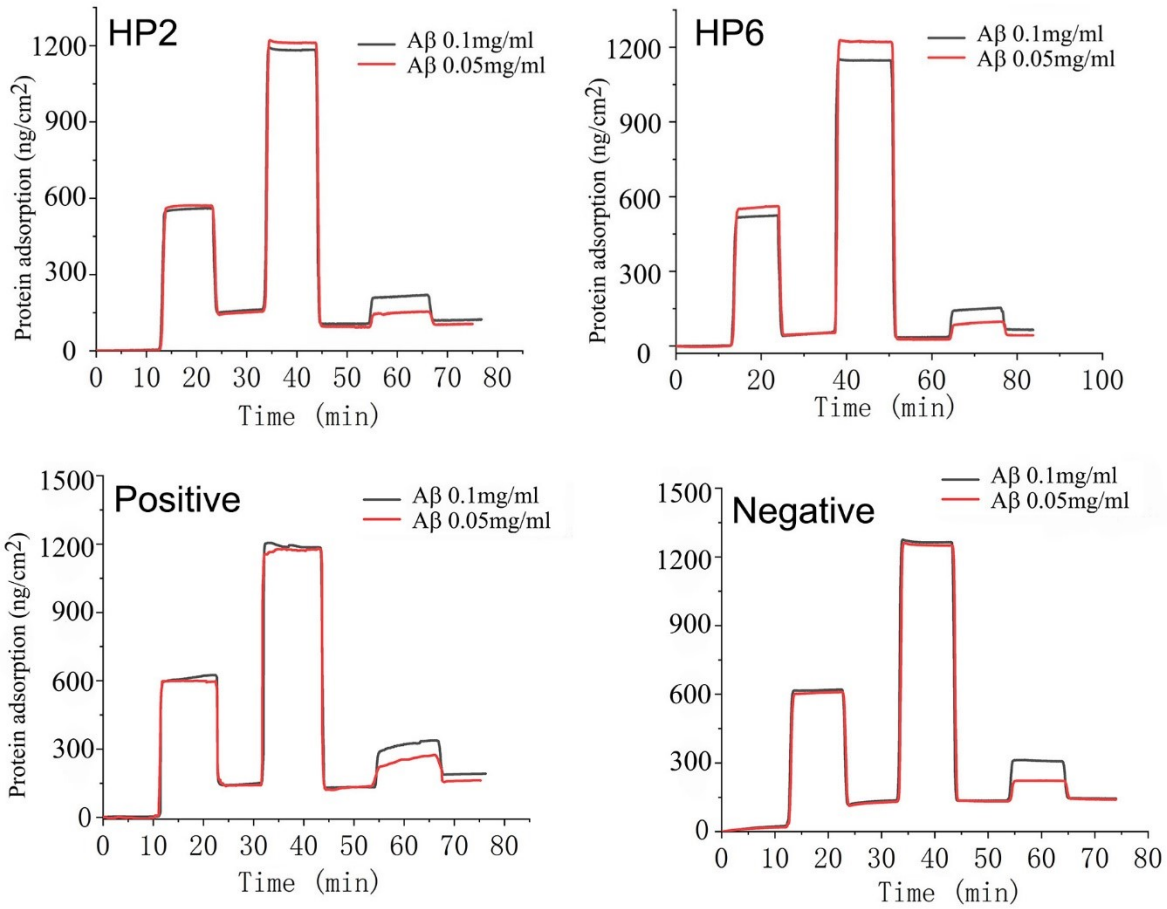


Figure S7. SPR sensorgrams for detecting the adsorption amount of Aβ using HP2- (CTLWWG), HP6- (GTVWWG), $A\beta_{27\text{-}32}$- (positive control), and PTRCGP- (negative control) coated SPR sensors.

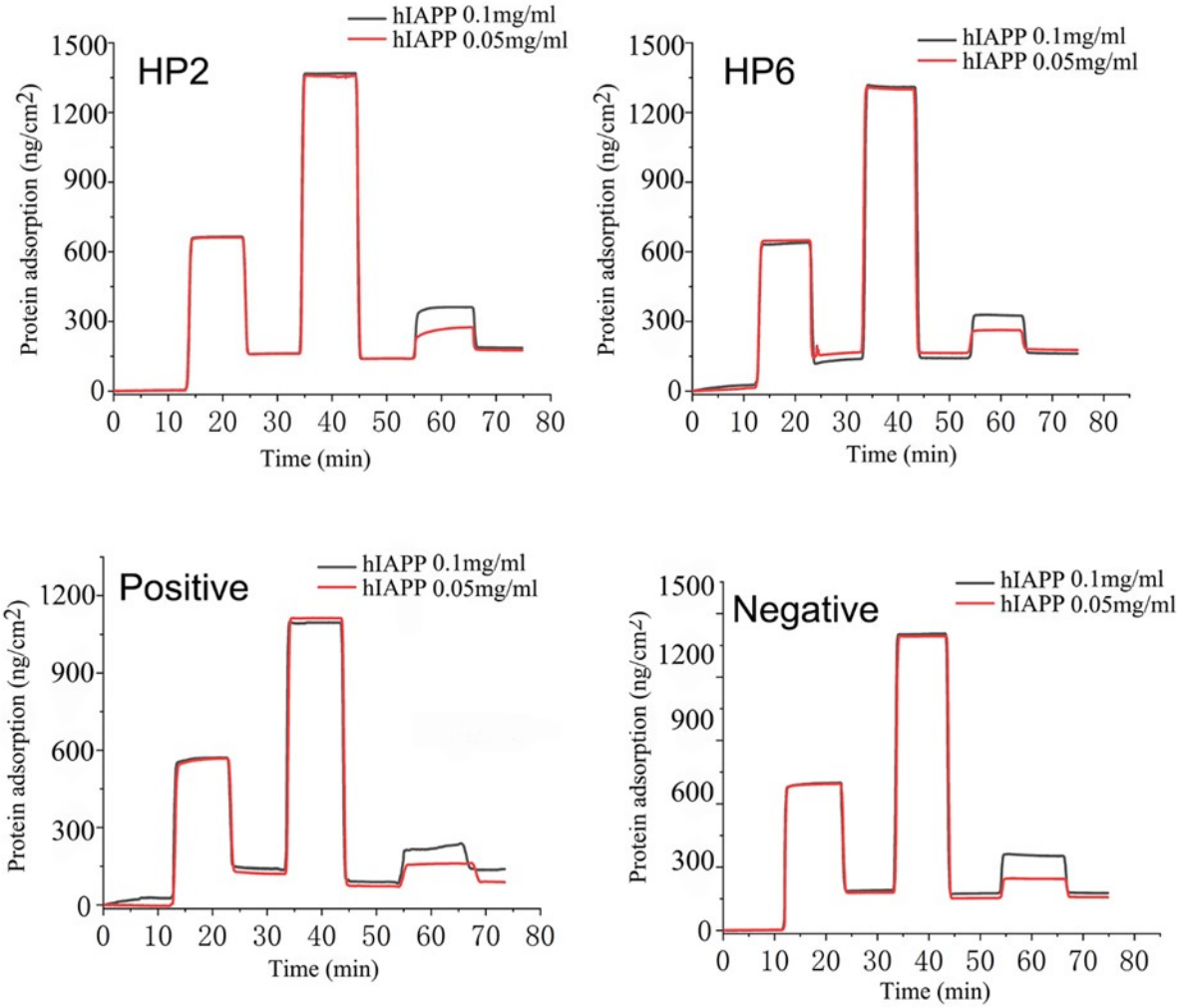


Figure S8. SPR sensorgrams for detecting the adsorption amount of hIAPP using HP2- (CTLWWG), HP6- (GTVWWG), $hIAPP_{1-6}$- (positive control), and PTRCGP- (negative control) coated SPR sensors.

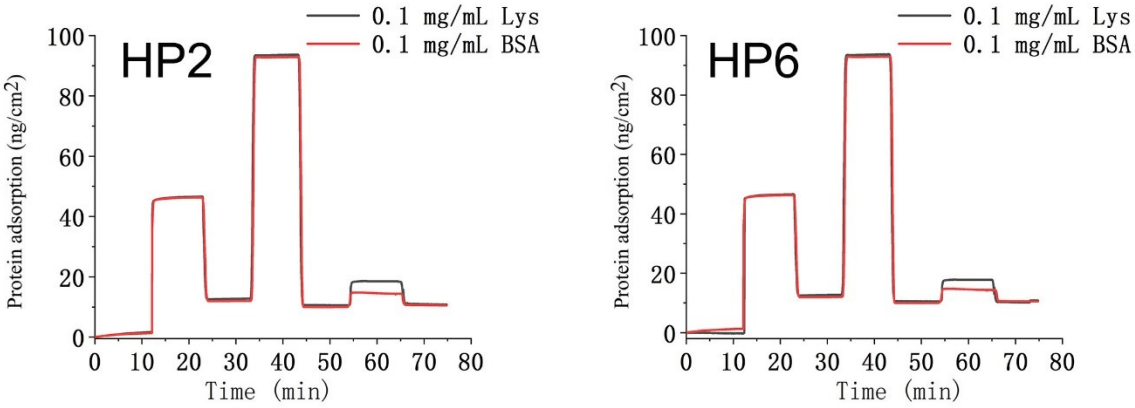


Figure S9. SPR sensorgrams for detecting the adsorption amount of Lysozyme and BSA using HP2- (CTLWWG), HP6- (GTVWWG) coated SPR sensors.